e), which were recrystallized from EtOH. In the case of no the oxidative post-treatment, pyrrolo(1,2-*b*)-pyridazines (IIa—g) were obtained directly by recrystallization of the crude cycloaddition products. The melting points and IR, NMR, and mass spectral data and microanalytical results of pyrrolo(1,2-*b*)pyridazines (IIa—g) are summarized in Table II.

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Synthesis of Active Forms of Vitamin D. IV.[1] Synthesis of 24,25- and 25,26-Dihydroxycholesterols[2]

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Recently, in the studies carried out on the activity of vitamin D, special attention has been paid to the metabolites, such as 25-hydroxy-, 1,25-, 24,25- and 25,26-dihydroxycholecalciferols. Among these compounds, it was found that 1,25-dihydroxycholecalciferol is the metabolically active form in the stimulation of intestinal calcium absorption and in the mobilization of calcium from bone, being considered as the hormonal form of vitamin D responsible for the maintenance of serum calcium at the expense of either bone or diet.[4] However, hitherto a complete determination of the biological significance of another metabolites, 24,25-dihydroxycholecalciferol (I)[5] and 25,26-dihydroxycholecalciferol (II)[6] have been hampered due to minuteness of available sample, obtained from the natural source. Therefore, it is of big need to find the method of synthesizing these compounds.

We describe in this paper on a synthesis of 24,25- and 25,26-dihydroxycholesterols which should be easily transformed to the corresponding vitamin D derivatives by the established procedures.[7] As described in the previous series, a characteristic aspect of our synthetic method is the ready availability of starting material. Thus, fucosterol which is abundant in brown algae is simply converted by our method[8] to desmosterol acetate (III), from which all the presently known active metabolites of vitamin D_3 could be obtained by rather brief processes.

Reaction of desmosterol acetate (III) with *m*-chloroperbenzoic acid gave in 54% yield, 24,25-epoxide,[9] which was then converted to 24ξ,25-diol (IVa),[10] mp 153.5—154.5°, by treatment with sulfuric acid in 79% yield. The same glycol (IVa) was obtained from III by oxidation with one equivalent of osmium tetroxide in a nearly quantitative yield. The structure of IVa was further characterized by transformation into diacetate (IVb).

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10) Determination of stereochemistry of C_{24} position is under investigation.

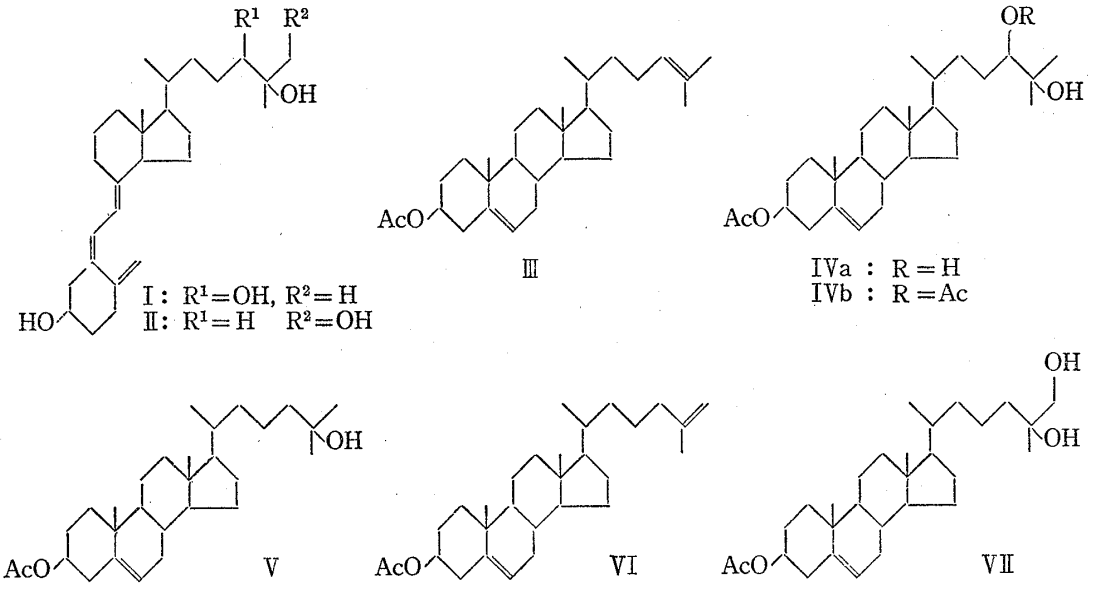

Chart 1

For the purpose of synthesis of 25,26-dihydroxy analogue (VII), 25-hydroxycholesterol acetate (V) synthesized[9] from III by oxymercuration-demercuration reaction was dehydrated with phosphorus oxychloride to give a 2:1 mixture of III and cholesta-5,25-dien-3β-ol acetate (VI).[11] An attempted isomerization of III into VI by reaction with iodine[12] was found fruitless on the basis of the negligible appearance of IR absorption at 890 cm^{-1} (exomethylene moiety). Separation of VI from III was effected by a carefull column chromatography on silica gel impregnated with silver nitrate. Oxidation of VI with osmium tetroxide gave 25ξ,26-glycol (VII), mp 169—171°, in a high yield. More conveniently, glycol (VII) was obtained from the olefinic mixture (III and VI), without separation, by treatment with osmium tetroxide, followed by separation of the resulting diol mixture (IVa and VII) by usual column chromatography on silica gel.

Experimental[13]

3β-Acetoxycholest-5-en-24ξ,25-diol (IVa)——a) To the solution of 3β-acetoxycholest-5-ene 24,25-epoxide[9] (418 mg) in tetrahydrofuran (80 ml), 1N H_2SO_4 (40 ml) was added and the mixture was stirred at room temperature overnight. Extraction with ether, washing with aq. $NaHCO_3$ and then water and evaporation of solvent gave a white amorphous (439 mg). Fractionation by column chromatography on silica gel (13 g) affored diol (IIIa) (331 mg) which was crystalized from methanol, mp 153.5—154.5°. $[\alpha]_D$ −13° ($CHCl_3$), NMR, 0.66 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.15 and 1.20 (6H, two s, 26, 27-CH_3), 2.02 (3H, s, acetyl), 3.34 (1H, m, 24-H), 4.6 (1H, m, 3-H) and 5.4 ppm (1H, m, 6-H). *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.34; H, 10.56.

b) To the solution of desmosterol acetate (III) (1.0 g)[8] in anhydrous ether (40 ml), OsO_4 (589 mg) was added and the mixture was stirred at room temperature for 19 hr. After evaporation of most of ether, the residue was dissolved in pyridine (60 ml). To this solution water (45 ml) and $NaHSO_3$ (2 g) was added and the mixture was stirred at room temperature for 17 hr. The reaction mixture was extracted with ether,

11) J.A. Svoboda and M.J. Thompson, *J. Lipid Res.*, **8**, 152 (1967).
12) N. Ikekawa, Y. Honma, N. Morisaki, and K. Sakai, *J. Org. Chem.*, **35**, 4145 (1970).
13) Melting points were determined on a hot stage using Yazawa Apparatus and uncorrected. Elemental analysis were carried out at the Laboratory of Organic Chemistry, Tokyo Institute of Technology. Infrared (IR) spectra were taken in CS_2 solution using Hitachi EPI-G2. Ultraviolet (UV) spectra were obtained with Hitachi ESP-3T in ethanol solution. Nuclear magnetic resonance (NMR) spectra were obtained on Varian T-60 in deuteriochloroform containing tetramethylsilane as internal reference. Chemical shifts were reported as ppm on the δ scale.

washed with 5% HCl, and then water. After the organic layer was dried over Na_2SO_4, the solution was concentrated under reduced pressure to give white crystal (1.0 g), crystallized from methanol, mp 151—152.5°.

3β,24ξ-Diacetoxycholest-5-en-25-ol (IVb)——Diol (IVa) (1.1 g) was treated with pyridine (35 ml) and acetic anhydride (12 ml) at room temperature overnight. Working up as usual gave diacetate (IVb) crystallized from acetone or *n*-hexane, mp 167.5—168°, NMR, 0.66 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.18 (6H, s, 26, 27-CH_3), 2.02 and 2.07 (6H, two s, acetyl), 4.7 (2H, m, 3- and 24-H), and 5.4 ppm (1H, m, 6-H). *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 74.22; H, 10.03.

3β-Acetoxycholesta-5,25-diene (VI)——Oxymercuration–demercuration reaction of III (1.93 g)[9] gave 25-hydroxycholesterol acetate (V), contaminated by *ca.* 10% (estimated from a glc analysis) of the unreacted III. This was treated with $POCl_3$ (2.4 ml) in pyridine (45 ml) at room temperature for 1 hr. The reaction mixture was extracted with ether, washed with 5% HCl and then water and dried over Na_2SO_4. Evaporation of solvent gave the olefinic mixture (1.90 g) which showed 2 spots on thin-layer plate of silica gel impregnated with $AgNO_3$ (10% wt) developed with benzene–*n*-hexane (85: 15). An analysis of this mixture by glc using all-glass capillary column (0.25 mm × 20 m)[14] coated with OV-17 at 260° equipped on Shimadzu-Gas-chromatograph 4BM-PTF revealed a 1: 2 ratio of VI to III. The mixture was applied on column of silica gel (50 g) impregated with $AgNO_3$ (10% wt).[15] Elution with *n*-hexane–benzene (7: 3) gave: fraction A, 670 mg, desmosterol acetate (III); fraction B, 560 mg, mixture of III and 3β-acetoxycholesta-5,25-diene (VI); fraction C, 260 mg, VI. Fraction C was crystallized from methanol–acetone to give VI, mp 106—107° (ref[11] 112—113°), IR (CS_2), 890 cm^{-1} (exomethylene), NMR, 0.67 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.7 (3H, broad s, 26-CH_3), 2.01 (3H, s, acetyl), 4.67 (2H, broad s, 27-methylene) and 5.4 ppm (1H, m, 6-H).

3β-Acetoxycholest-5-ene-25ξ,26-diol (VII)——a) To the solution of diene (VI) (236 mg) in ether (20 ml), OsO_4 (141 mg) was added and the mixture was stirred at room temperature overnight. Ether was evaporated and the residue was treated with $NaHSO_3$ (0.5 g), water (12 ml) and pyridine (15 ml) at room temperature for 3.5 hr. Extraction with ether, washing with 5% HCl and then water, drying over Na_2SO_4 and evaporation of solvent gave a white amorphous which was purified by column chromatography on silica gel to give 25,26-diol (VIIa) (170 mg), crystallized from methanol or ethanol–water, mp 169—171°, $[\alpha]_D$ −12° ($CHCl_3$), NMR, 0.67 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.15 (3H, s, 26-CH_3), 2.02 (3H, s, acetyl), 3.42 (2H, -CH_2OH), 4.6 (1H, m, 3-H) and 5.35 ppm (1H, m, 6-H). *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50, Found: C, 75.13; H, 10.43.

b) A mixture (585 mg) of olefine III and VI (the ratio undetermined) was treated with OsO_4 (351 mg) in ether (15 ml) at room temperature overnight. The extract with ether was treated with $NaHSO_3$ (1.5 g), water (15 ml) and pyridine (30 ml) at room temperature for 3 hr. Pyridine was evaporated off *in vacuo* and the residue was dissolved in ether, washed with water and dried over Na_2SO_4. Evaporation of solvent gave a white amorphous (619 mg) which was fractionated by column chromatography on silica gel. Elution with benzene–ethyl acetate (9: 1) gave 24,25-diol (IVa) (257 mg), mixture of IVa and VII (150 mg) and 25,26-diol (VII) (130 mg).

14) N. Ikekawa, M. Morisaki, and M. Nakane, unpublished.

15) Prepared as follows: silver nitrate (30 g) was dissolved in refluxing ethanol (400 ml) and, to this solution, silica gel (270 g) was added. The mixture was thoroughly shaken and then ethanol was evaporated off *in vacuo*. The residue was dried in a evacuated desiccator.